Running title: autonomic dysfunction and ACR

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Cardiovascular autonomic dysfunction predicts increasing albumin excretion in type 1 diabetes

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ABSTRACT

Objectives: To determine the potential role of cardiovascular autonomic dysfunction in the development of renal complications in young people with type 1 diabetes (T1D).

Methods: In this prospective study, 199 children and adolescents recruited to the Oxford Regional Prospective Study underwent assessment of autonomic function ~5 years after diagnosis, and were subsequently followed with longitudinal assessments of HbA_{1c} and urine albumin-creatinine ratio (ACR) over 8.6 ± 3.4 years. Autonomic function was assessed with 4 standardized tests of cardiovascular reflexes: heart rate (HR) response to (i) Valsalva Maneuver, (ii) deep breathing, and (iii) standing, and (iv) blood pressure (BP) response to standing. Linear mixed models were used to assess the association between autonomic parameters and future changes in ACR.

Results: Independent of HbA_{1c}, each SD increase in HR response to Valsalva Maneuver predicted an ACR increase of 2.16% [95% CI: 0.08; 4.28] per year (p=0.04), while each SD increase in diastolic BP response to standing predicted an ACR increase of 2.55% [95% CI: 0.37; 4.77] per year (p=0.02). The effect of HR response to standing on ACR reached borderline significance (-2.07% [95% CI: -4.11; 0.01] per year per SD increase, p=0.051).

Conclusions: In this cohort of young people with T1D, enhanced cardiovascular reflexes at baseline predicted future increases in ACR. These results support a potential role for autonomic dysfunction in the pathogenesis of diabetic nephropathy.

Key words: autonomic dysfunction, albumin excretion, type 1 diabetes, adolescents

INTRODUCTION

Subclinical autonomic neuropathy is a common complication of type 1 diabetes (T1D), which has been observed as early as 2 years after T1D diagnosis [1]. A recent systematic review has reported a variable prevalence of abnormal cardiovascular nerve function tests in young people with T1D, ranging from 16 to 75% [2].

Autonomic dysfunction has been proposed as a pathogenic mechanism which may underlie future renal and cardiovascular complications in the general population and in people with T1D [3–5]. Cross-sectional studies indicate that impaired autonomic function, as documented by conventional cardiovascular reflex tests or spectral analysis of resting electrocardiograms, is associated with renal complications of T1D [6,7]. However, human clinical data are limited and the best evidence for causality comes from preclinical models, whereby renal denervation increased albumin excretion rates (AER) in streptozotocin-induced diabetic rats [8].

Extensive evidence indicates that increases in urinary albumin excretion, even within the normal range, predict renal and cardiovascular disease (CVD) risk in the normal population as well as in people with T1D [9,10]. Increased albumin-creatinine ratio (ACR) within the normal range has been found to predict 85% of adolescent patients who will subsequently develop microalbuminuria as young adults [11]. In addition, in adolescents with T1D, an ACR in the top 30% of the normal range is associated with early signs of cardiovascular disease, such as increased arterial stiffness and aortic intima-media thickness [12,13].

Longitudinal data exploring the relationship between autonomic dysfunction and subsequent changes in urinary albumin excretion could be valuable in determining

the contribution of autonomic dysfunction to the pathogenesis of renal and cardiovascular complications of T1D. However, to date only two studies have been reported [14,15] and they showed that smaller resting pupil diameter [15] and reduced heart rate response to deep breathing [14] at baseline increased the risk of developing micro- or macroalbuminuria during follow-up. One of these studies involved an adolescent population, but it was limited by a high rate of loss of subjects during follow-up (41% of the original cohort) [2,15]. The other study was based on an adult population with T1D, with a 13-year duration of diabetes, and a high prevalence (50%) of micro- or macroalbuminuria at baseline [14].

The aim of the present study was to assess the association between cardiovascular autonomic dysfunction and subsequent changes in urinary albumin excretion in a cohort of young people with childhood-onset T1D recruited and followed in the Oxford Prospective Regional Study (ORPS).

MATERIALS AND METHODS

Recruitment and follow-up

ORPS is a large, well-characterized, population-based inception cohort of childhood-onset T1D patients, recruited at diagnosis and followed thereafter with annual standardized assessments [16,17]. The study methods have been reported in detail elsewhere [11,16–18]. Briefly, children and adolescents with T1D were recruited during a 10-year period, between 1986 and 1996, from the St. Bartholomew's Oxford diabetes register. T1D patients had to be less than 16 years old at the time of diagnosis, and were approached within 3 months of diagnosis. Ninety-one percent (n=527) of eligible children were recruited at a mean age of 8.8 years and were followed annually thereafter. The overall dropout rate for the ORPS cohort has been 9.6%. The study received ethical approval from district ethics committees. Written consent was obtained from parents, and verbal assent was obtained from children.

213 participants agreed to have a one-off autonomic assessment approximately 5 years after T1D diagnosis. Of these, 14 participants were excluded due to incomplete data, and the remaining 199 represent the study population for the present study.

Annual assessments

Annual assessments included anthropometric measurements (height, weight, BMI), collection of blood samples for the measurement of HbA_{1c} and collection of urine samples for the assessment of ACR. Due to the variability in urine ACR, 3 consecutive first-void early morning urine samples were collected from each participant, and the geometric mean of the ACR measurements was calculated. All

biochemical measurements were performed centrally. HbA_{1c} was measured initially using electrophoresis and then, after 1992, using high performance liquid chromatography. Albumin and creatinine were measured using double antibody enzyme linked immunosorbent assay (ELISA) and the modified Jaffe method respectively. The relationship between urine ACR and AER has been characterized in this cohort [18]. As in previous studies [11,16,17,19], microalbuminuria was defined as an ACR of 3.5-35mg/mmol in males and 4.0-47mg/mmol in females. Macroalbuminuria referred to an ACR of >35mg/mmol in males and 47mg/mmol in females. The ACR was not normally distributed and was $\log_{10}$ transformed.

Autonomic assessment

The autonomic assessment comprised 4 standard tests of cardiovascular reflexes, performed following the methods described by Ewing and colleagues [20]. These tests assessed the (i) heart rate (HR) response to Valsalva Maneuver, (ii) HR response to deep breathing, (iii) HR response to standing, and (iv) blood pressure (BP) response to standing, and were performed in this order. Autonomic parameters summarizing the result of each cardiovascular reflex test were calculated from raw measurements, as follows:

(i) HR response to Valsalva Maneuver: Longest RR after Valsalva Maneuver ÷ Shortest RR before Valsalva Maneuver;

(ii) HR response to deep breathing: 60/Shortest RR – 60/Longest RR, with RR interval in seconds;

(iii) HR response to standing: Longest RR ÷ Shortest RR;

(iv) BP response to standing: Systolic BP (SBP) response= SBP standing - SBP lying; Diastolic BP (DBP) response= DBP standing - DBP lying

All ratios were $\log_{10}$ transformed for further analysis to maintain symmetry along a linear scale.

Statistical analysis

Linear mixed models (random coefficient models) were used to maximize the statistical power of the repeated outcome measurements made over time in the longitudinal study design [21], while adequately accounting for the correlation between measurements [22,23].

A linear mixed model was created including time (since autonomic assessment) as a first level predictor, and the following covariates: sex, duration of diabetes, age at autonomic assessment, mean HbA_{1c} during duration of follow-up (after autonomic assessment). An unstructured covariance was used and parameter estimation performed using the maximum likelihood method. To determine if random effects, i.e. unexplained variation, in baseline ACR and its rate of change over time needed to be modeled, the -2 Log Likelihood statistic was compared between alternative models using the χ^2 test [24]. The best fit was obtained with both random intercepts and random slopes included, yielding an Akaike's Information Criterion (AIC) of 598.39. Separate models were then created, each including one of the five autonomic parameters measured, unless otherwise stated. Autonomic parameters were transformed into Z-scores before inclusion in the models, to facilitate comparison of their relative effect sizes. Z-scores were calculated using the formula $(x-\mu_x)/SD_x$, where x refers to the autonomic parameter under consideration, and μ and SD refer to the mean and standard deviation respectively. Only ACR and HbA_{1c}

measurements made after autonomic assessment were used, as it is the period after autonomic assessment which is the study period under consideration.

SPSS Version 23 (IBM Corp., Armonk, NY) was used for all analyses, and a p-value of 0.05 used as the cut-off for statistical significance. Normality was determined graphically. All values are given as mean ± SD unless otherwise specified.

RESULTS

The clinical and autonomic characteristics of the 199 study participants are shown in Table 1. These 199 participants did not differ from the remainder of the ORPS cohort in terms of sex distribution (female: 47.2% vs 44.2%), age at diagnosis (median [interquartile range]: 9.25 [5.94-1180] vs 9.73 [5.01-12.42] years), mean HbA_{1c} (9.69 ± 1.38 vs 9.89 ± 1.58% or 82.4 ± 15.1 vs 84.6 ± 17.3 mmol/mol), mean log_{10}ACR (0.023 ± 0.282 vs 0.031 ± 0.326).

Longitudinal profile of Urine ACR

At the time of autonomic function assessment, only 14 participants had ACR measurements in the micro- or macroalbuminuric range. During follow-up, 57 participants showed ACR in the micro- or macroalbuminuric range, with 8 developing macroalbuminuria. Using linear mixed models with time as the only covariate, the longitudinal profile of urine ACR was explored. As shown in Figure 1, participants who developed micro- or macroalbuminuria demonstrated an increase in ACR with time (9.94% [95% CI: 3.21 – 17.11] per year, p=0.004), while participants who remained normoalbuminuric demonstrated a small decrease in ACR with time (-2.28% [95% CI: -3.24 – -1.32] per year, p<0.001).

Effect of Cardiovascular Reflexes on Urine ACR

The value of cardiovascular reflex tests performed at baseline in predicting subsequent changes in urine ACR was tested using linear mixed models, adjusting for sex, duration of diabetes, age of assessment and mean HbA_{1c} during follow-up.

Of the examined autonomic parameters, 2 displayed a significant relationship with the rate of change of ACR: HR response to Valsalva Maneuver (2.16% [95% CI: 0.08; 4.28] per year per SD increase, p=0.041), and DBP response to standing (2.55% [95% CI: 0.37; 4.77] per year per SD increase, p=0.022) (Figure 2). The effect of HR response to standing reached borderline significance (-2.07% [95% CI: -4.11; 0.01] per year per SD increase, p=0.051). The HR response to standing was not determined by the maximum heart rate response (p=0.75), but instead by the longest RR interval (p<0.001), indicating persistence of the initial cardio-acceleratory response. In these models, the effect sizes of autonomic parameters were of comparable magnitude to that of HbA_{1c}, i.e. 5.55-5.94% per year per SD increase (p<0.001) or 3.86-4.13% per year per % increase.

To test if the predictive effect of HR response to Valsalva Maneuver, DBP response to standing and HR response to standing were independent and thus additive, these autonomic parameters were introduced into the same linear mixed model together with the aforementioned covariates. HR response to standing displayed a significant relationship with the rate of change of urine ACR (-2.65% [95% CI: -4.85 – -0.040] per year per SD increase, p=0.022). However, HR response to Valsalva Maneuver was of borderline significance (2.30% [95% CI: -0.003 – 4.66] per year per SD increase, p=0.050), and there was no significant effect of DBP response to standing (1.23% [95% CI: -1.05 – 3.56] per year per SD increase, p=0.29).

On bivariate analysis of the 3 autonomic parameters, the only significant correlation was between HR response to Valsalva Maneuver and DBP response to standing (r=0.216, p=0.004). To assess the influence of colinearity between HR response to Valsalva Maneuver and DBP response to standing on these results, the latter was

removed from the model. This resulted in the effect of HR response to Valsalva Maneuver reaching statistical significance (2.16% [95% CI: 0.12 – 4.24] per year per SD increase, p=0.038), and a largely unchanged effect of HR response to standing (-2.40% [95% CI: -4.43 – -0.33] per year per SD increase, p=0.023) (Table 2).

Similarly, when HR response to Valsalva Maneuver was removed from the model, the effect size of HR response to standing remained similar (-2.28% [95% CI: -4.50 – -0.015] per year per SD increase, p=0.049), while the effect size of DBP response to standing increased but still did not reach significance (1.66% [95% CI: -0.59 – 3.96] per year per SD increase, p=0.15).

DISCUSSION

In this study, we report an association between early signs of cardiovascular autonomic dysfunction and increasing urine ACR in young people with childhood-onset T1D. To our knowledge, this is the first such report in a young population with T1D predominantly normoalbuminuric at baseline.

The longitudinal profile of urine ACR in this cohort is in line with earlier observations suggesting that only certain patients with T1D are susceptible to developing diabetic nephropathy [17,25]. ACR only increased longitudinally in the subpopulation of participants who eventually developed micro- or macroalbuminuria, and instead was stable or even decreased in participants who remained normoalbuminuric during follow up.

In this study, we showed that autonomic parameters derived from standard tests of cardiovascular reflexes performed at baseline, were predictive of subsequent increases in ACR with time, independent of HbA_{1c}. More specifically, longitudinal increases in ACR were predicted by an enhanced HR response to the Valsalva Maneuver as well as an enhanced DBP response to standing. There was also an association with HR response to standing that reached borderline significance. Thus, early cardiovascular autonomic dysfunction in the form of enhanced cardiovascular reflexes is associated with subsequent longitudinal increases in ACR.

This pattern of autonomic dysfunction is consistent with enhanced sympathetic tone relative to parasympathetic or vagal tone [26,27], as has been also observed in T1D populations using spectral analysis of resting electrocardiograms [28].

Relative sympathetic overactivity may represent an important mechanism by which renal injury occurs. In the large population-based Atherosclerosis Risk in Communities study, a relative increase in sympathetic tone as identified by spectral analysis of electrocardiograms, was associated with increased risk of chronic kidney disease-related hospitalizations, even after adjusting for diabetes status, fasting plasma glucose and insulin, in addition to other covariates [29]. In addition, other clinical phenomena associated with renal injury in populations with and without diabetes may have their basis in relative sympathetic overactivity. Examples include the non-dipper phenomenon [30,31], as well as orthostatic hypertension [32,33].

A potential study limitation might be the methodology used to assess autonomic dysfunction. Although the selected tests of cardiovascular reflexes are well-validated and clinically applicable [34–36], they reflect autonomic function only at a certain time of the day, instead of a 24-hour assessment of autonomic function. Recent studies have mainly used time and frequency domain measures of heart rate variability to characterize cardiac autonomic function in patients with diabetes, and these measures are thought to be more reproducible and better tolerated by patients. However, some previous studies reported a good correlation between results of cardiovascular reflexes and time and frequency measures [37], and similar associations with microalbuminuria [38]. An additional limitation of the present study could be related to the the inclusion of autonomic parameters as interval variables in the multivariable analyses, thus providing little guidance as to which values of autonomic parameters are considered abnormal and might warrant greater clinical attention. In addition, it needs to be acknowledged that glycemic control in this historical population, mainly on twice-daily insulin regimen, was well-above the

recommended values for adolescents and this might have influenced the study findings, and limits the applicability of the study findings to populations of adolescents with T1D and better glycemic control.

In conclusion, in a predominantly normoalbuminuric cohort of young people with childhood-onset T1D, we demonstrated that enhanced cardiovascular reflexes predicted future increases in urine ACR. These suggest that detection of autonomic dysfunction early in the course of T1D may enable the identification of a subpopulation of patients at increased risk of microalbuminuria and diabetic nephropathy, and who, *a priori*, may benefit from earlier interventions.

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Conflict of Interests/Disclosures: None

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Tables

Table 1. Clinical and autonomic characteristics of study participants

Study Parameters	
N	199
Female (%)	47.2
Age at diagnosis (years)	9.25 (5.94 – 11.80)
Duration of diabetes at autonomic assessment (years)	5.17 ± 0.35
Age at autonomic assessment (years)	14.16 (11.02 – 17.02)
Duration of follow-up after autonomic assessment (years)	8.59 ± 3.39
Mean HbA_{1c} during entire follow-up (%) [mol/mol]	9.69 ± 1.38 [82.4 ± 15.1]
Mean HbA_{1c} after autonomic assessment (%) [mol/mol]	9.63 ± 1.42 [82.0 ± 15.5]
Mean log_{10}ACR during entire follow-up	0.023 ± 0.282
Mean log_{10}ACR after autonomic assessment	0.085 ± 0.347
HR response to Valsalva Maneuver	1.76 ± 0.40
HR response to deep breathing (bpm)	28.85 ± 8.19
HR response to standing (30:15 ratio)	1.24 ± 0.20
HR response to standing	1.38 ± 0.20
Systolic BP response to standing (mmHg)	-0.43 ± 9.42
Diastolic BP response to standing(mmHg)	3.06 ± 9.86

Average values are given as mean ± SD if normally distributed or median (IQR) if not.

ACR: albumin creatinine ratio, HR: heart rate, BP: blood pressure.

Table 2. Linear mixed model: Effect of autonomic parameters and other covariates on change of longitudinal ACR (% change per year)

Variable	Effect Size	95% CI	p-value
Female sex	0.85	-3.18; 5.05	0.68
Duration of diabetes (years)	-2.63	-8.14; 3.20	0.37
Age at assessment (years)	0.12	-0.42; 0.67	0.66
Average HbA_{1c} (%)	3.91	2.36; 5.48	<0.001
HR response to Valsalva Maneuver (Z score)	2.16	0.12; 4.24	0.038
HR response to standing (Z score)	-2.40	-4.43; -0.33	0.023

CI: confidence interval; HR: heart rate

Figure Legends

Figure 1. Longitudinal profile of urine albumin-creatinine ratio (ACR). Urine ACR increased with time in participants who developed micro- or macroalbuminuria (MA) ($p=0.004$), but decreased in participants who remained normoalbuminuric (no MA) ($p<0.001$).

Figure 2. Effect of individual autonomic parameters on the rate of change of longitudinal ACR. Age at autonomic assessment, duration of diabetes, mean HbA_{1c} during follow-up and sex were adjusted for. Effect sizes of the individual autonomic parameters were standardized to their SDs. HR: heart rate, BP: blood pressure. * refers to $p<0.05$. † refers to $p=0.051$.

Figure 1

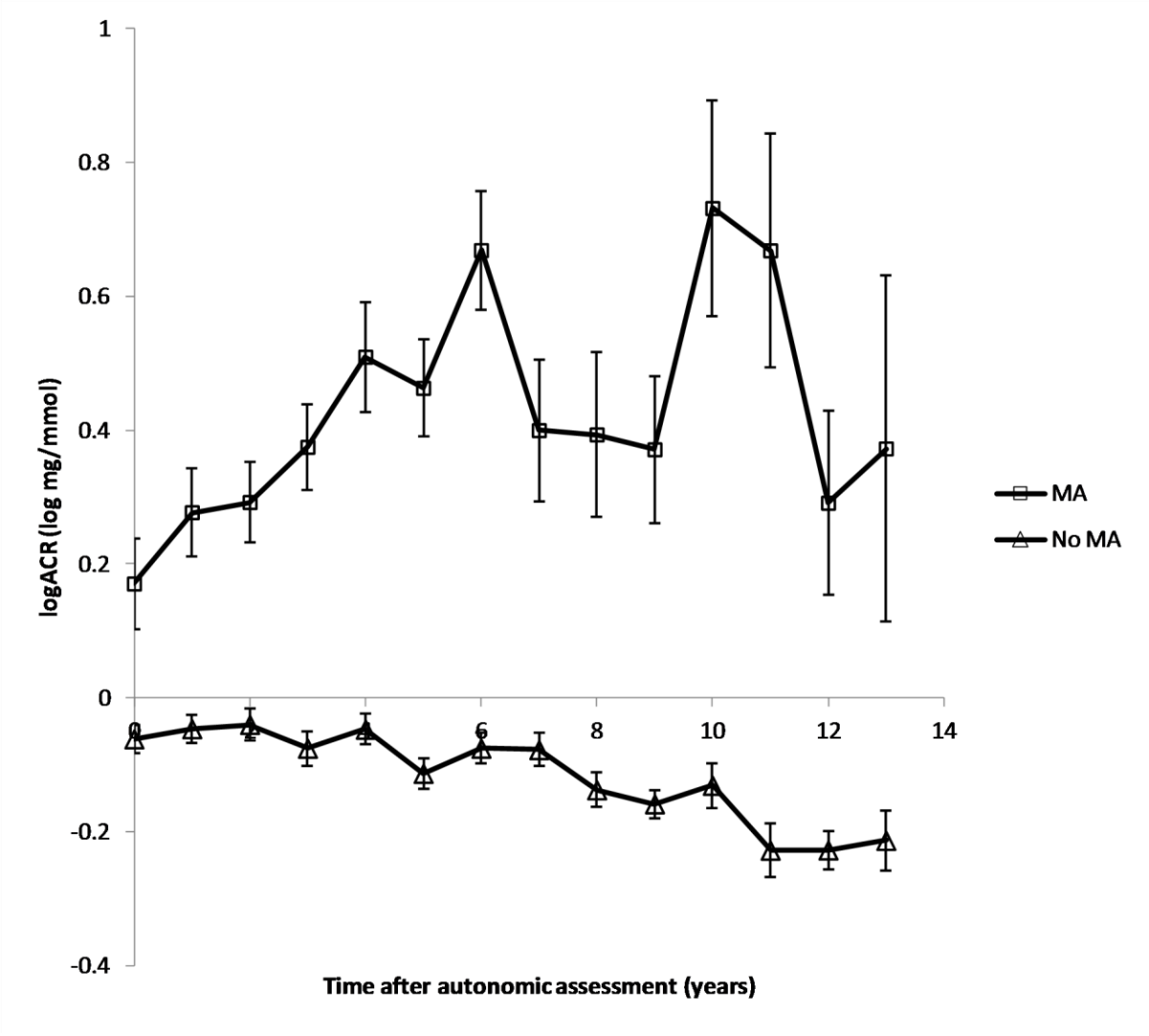
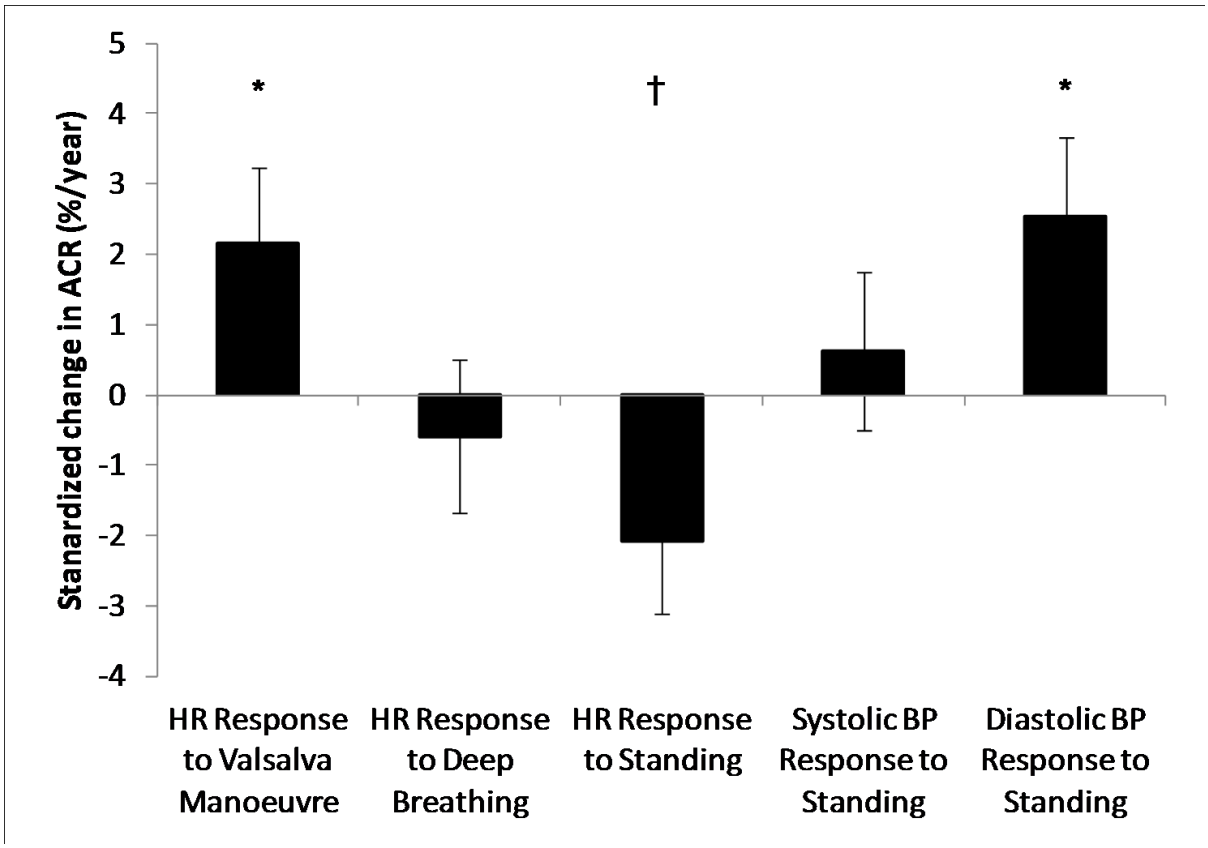

1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
logACR (log mg/mmol)
0
6
8
10
12
14
Time after autonomic assessment (years)
MA
No MA

Figure 2


Stanardized change in ACR (%/year)
5
4
3
2
1
0
-1
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-3
-4
*
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*
HR Response to Valsalva Manoeuvre
HR Response to Deep Breathing
HR Response to Standing
Systolic BP Response to Standing
Diastolic BP Response to Standing